



## **Microbial community stratification in Membrane-Aerated Biofilm Reactors for Completely Autotrophic Nitrogen Removal**

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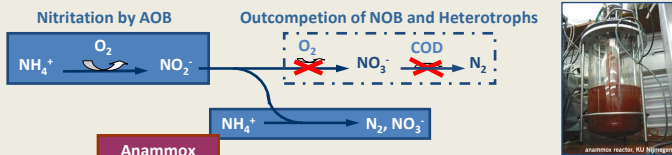
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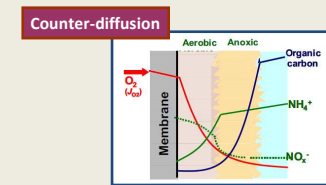
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## 1. Introduction

Anaerobic Ammonium Oxidizers (Anammox): A relatively new paradigm in Nitrogen removal from wastewater



But why using two reactors when one is enough? -> Biofilms



### Advantages

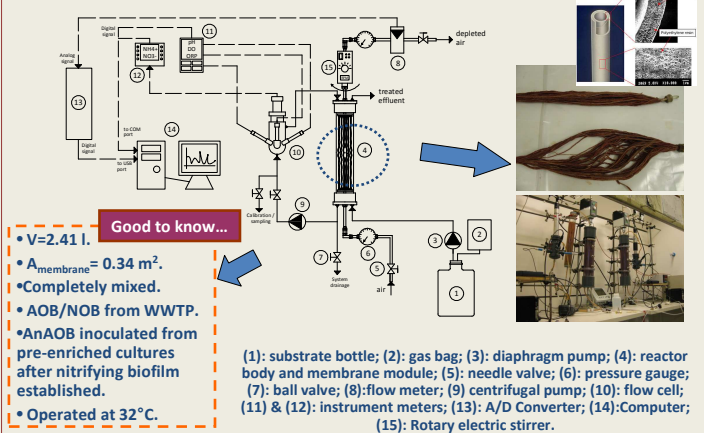
- Compact configuration.
- Lower N<sub>2</sub>O production (NO<sub>2</sub><sup>-</sup> is produced and consumed in-situ).

### Disadvantages

- Need of a carrier material.
- Difficulty of control (thickness, O<sub>2</sub> supply, microbial selection...).

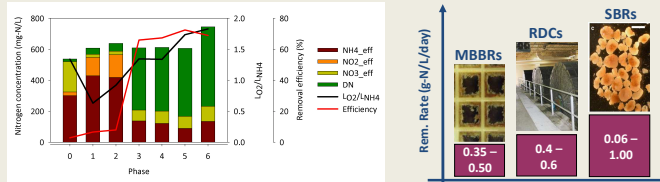
## 2. Reactor setup

Our biofilm develops on hollow-fibre bubbleless aeration membranes. More in detail, the setup consists of



## 3. Performance

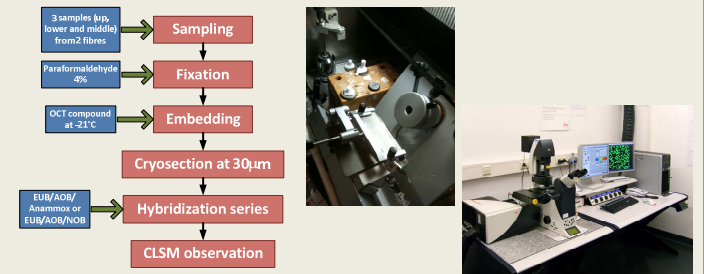
Through sequential aeration regimes and L<sub>O2</sub>/L<sub>NH4</sub> tuning to 1.73 the reactor performance increased considerably since Anammox inoculation



- Observed removal rates up to 0.77 g-N/L/day (5.5 g-N/m<sup>2</sup>/day, 70% of the N-load). The Anammox process is expected to remove most of it.
- NOB produces 30% of the total NO<sub>3</sub><sup>-</sup>.

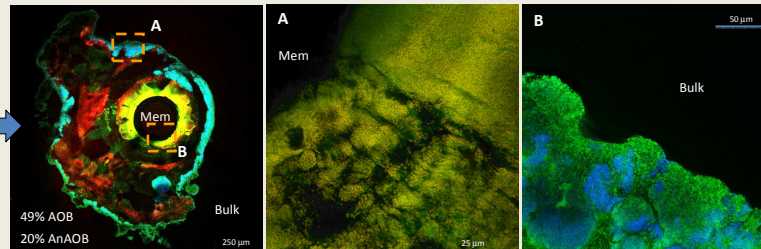
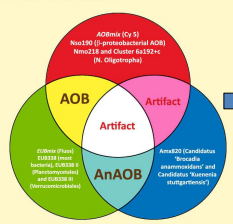
## 4. Materials and methods

Fluorescent In-Situ Hybridization was performed after reactor shutdown (day 470, 0.7 g-N/L/day) to study the microbial community in the reactors



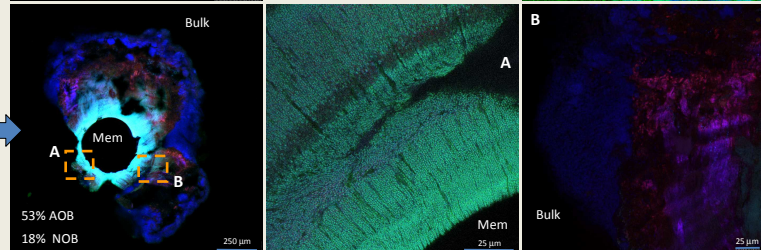
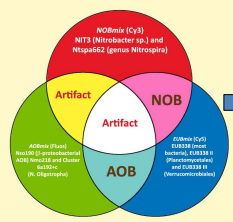
## 5. Microscopic observation & quantification - Conclusions

### Probe combination 1



Complete section (x10) of a fibre targeted for EUB, AOB and AnAOB, with details of the internal part (A) and the external part (B) of the biofilm.

### Probe combination 2



View of a section targeted for AOB, NOB and EUB and the respective details of the internal (A) and the external (B) parts, with the same magnifications used before. Mem: Membrane; Bulk: Bulk liquid. The results of quantification are expressed in percentage respect to the general probe area obtained in the complete section pictures.

- Results proved the possibility of engineering biofilm structures for autotrophic nitrogen removal taking advantage of DO gradients inside the biofilm
- AOB grew mainly in aerobic regions, close to the membrane in the biofilm core. Communities developed in areas with high cell densities and tended to grow in radial structures with streptobacilli shape
- AnAOB grew in the perimeter of the biofilm structure, where anaerobic conditions prevailed. Since nitrite was produced in-situ by AOB, AnAOB grew in clusters pointing the internal part of the biofilm
- NOB developed mainly during the reactor start-up, before Anammox inoculation, and were mainly located in the transient zone of the biofilm, together with other non-identified bacteria (most likely heterotrophic).
- Image quantification using DAIME showed that AOB were the most abundant population while AnAOB only covered 20% of the biofilm area.